Physiological Determinants of Invasive Success: Linking Distribution Patterns to Metabolic Physiology in Native and Invasive Blue Mussels (genus *Mytilus*)

by

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In the early 20th century, a blue mussel species from the Mediterranean invaded the California coast and subsequently out-competed the native species south of Monterey Bay. Like other invasive species, *Mytilus galloprovincialis* has physiological traits that make it successful in habitats formerly occupied by the native *M. trossulus*, namely its adaptation to warm sea surface temperatures. This study looks at the current genotype distributions and enzymatic activities of field-acclimatized mussels within the hybrid zone where the species co-occur as well as mussels that have been acclimated for four weeks to different temperature and salinity conditions. In the field-acclimatized and laboratory-acclimated mussels, the native species exhibited significantly higher enzyme rates, which may reflect an evolutionary adaptation to compensate to low habitat temperatures. Indeed, the results of the laboratory acclimation indicate that these differences are genetically based. Whether an acclimation capacity exists may require even longer-term acclimation to different temperatures. Current findings suggest that the further spread of the invasive species is likely to be governed in large measure by the potentially counteracting effects of rising temperatures, which would favor the northerly spread of *M. galloprovincialis*, and increased winter precipitation, which would favor the persistence of *M. trossulus*. However, the success of *M. galloprovincialis* during acclimation to ‘dilute’ salinity (25 ppt) suggests that the invasive species can tolerate a greater salinity range than previously thought. Thus, further investigation is needed to build a comprehensive predictive model of the movement of *M. galloprovincialis* and the hybrid zone along the California coast.
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Chapter 1: Introduction

Why Study Marine Invasions?

Biological invasions are one of the most significant drivers of global environmental change and can come with profound ecological and economic costs. The estimated annual expenditure on controlling and managing marine and terrestrial invasive species in the United States is more than $138 billion (Pimentel et al., 2005). Ever-increasing rates of human activities such as international trade, travel, and transport have facilitated the introduction of many invasive species worldwide. Invasive species typically have physiological characteristics such as rapid growth rate, large reproductive output, and high environmental stress tolerance that allow them to out-compete native species for space and resources, thereby threatening biodiversity, ecosystem function, and habitat quality. Therefore, studying invasive species is essential in order to gain control of and prudently manage our ecological and economic resources.

Marine invasive species pose significant threats to ocean health and overall global biodiversity. The health of the oceans and humans’ economic and physical wellbeing are directly tied to the balance maintained within marine ecosystems. Given that marine invasive species threaten to disrupt ecological balances, a considerable amount of research has focused on the factors underlying invasive processes. The more fully we understand the mechanisms that determine the success of invasions, the better we will be able to predict future impacts of invasions on marine ecosystems and thus be able to interrupt these processes at an earlier, more manageable stage. Invasions in marine ecosystems are predicted to increase as climate warms (Stachowicz et al., 2002; Hilbish et al., 2010), so understanding the underlying causes of invasions is especially significant.
in the context of on-going global change. Currently, the scientific community has only a limited understanding of the links between the diverse processes comprising global change and invasive success. However, for my two study species, strong linkages between variations in temperature and salinity and distribution patterns suggest that these organisms could serve as an important model for the study of the effects of global change on marine invasions (Braby and Somero, 2006a; Hilbish et al., 2010).

Early detection and rapid response to invasive species is key to controlling invasions and limiting their potentially devastating ecological and economic effects. Furthermore, scientific understanding of the geographical extent and environmental impact of invasions is crucial for assessing the appropriate management steps. Hence, scientific information is instrumental in the formulation and implementation of effective policies and practices in order to constrain invasive species and cap their damages.

**An Invasive Species in California Waters: Mytilus galloprovincialis**

*Mytilus galloprovincialis* (Lamarck 1819), also known as the Mediterranean blue (or bay) mussel, was accidentally introduced to the Pacific Coast of North America in the early twentieth century. Since this time, it has successfully invaded the subtidal and intertidal habitats occupied formerly and currently by its native blue mussel congener, *M. trossulus* (Gould 1850). Currently, *M. galloprovincialis* is the dominant blue mussel at latitudes between Baja California and Monterey Bay. North of Cape Mendocino, *M. trossulus* is dominant. Between Monterey Bay and Cape Mendocino (latitudes 36.63°N–40.5°N), both species and their hybrids are present in what is termed a ‘mosaic-hybrid’ zone (Braby and Somero, 2006a) (Figure 1.1).
As is the case when any non-native species is introduced and takes hold in a new
habitat, the scientific community seeks to better understand the adaptive properties that
have enabled it to thrive as well as the impact the invasion is currently having and is
likely to have on the ecosystem it has entered.

**Thesis Goals**

This study is a physiological comparison of field-acclimatized and laboratory-
acclimated specimens of *M. galloprovincialis*, *M. trossulus*, and their hybrids aimed at
gaining insight into their differential thermal and osmotic adaptive properties in order to
contribute to building a mechanistic understanding of the *M. galloprovincialis* invasion

*Figure 1.1:* Map of California mosaic-hybrid zone. Distribution ranges of *M. trossulus* shown in white, hybrids in gray, and *M. galloprovincialis* in black.
and predict the future movement of the invasive species. My work focuses on differences in metabolic capacity between the two species and between the two parental species and their hybrids. Studies of the hybrids reflect the importance of determining whether a form of ‘hybrid vigor’ (heterosis) is present, which might allow the hybrids to function over wider ranges of temperature and salinity than the two parental species. My intention is to contribute data that will help build a mechanistic (physiological) understanding of the differences between the native and invasive species. Eventually, this type of information can be used to predict the further spread of invasive mussel species in the context of a changing climate characterized by rising temperatures and altered precipitation patterns. Building a mechanistic model that could reliably predict the future rates and patterns of invasive species movement would enable cost-benefit analysis to be conducted to determine if extirpative measures would be fiscally prudent.

Specifically, this study attempts to gain insight into the abilities of the native and invasive species to sustain metabolic function under different conditions of temperature and salinity. In particular, the ability of *M. galloprovincialis* to function at the temperatures and salinities of the Eastern Pacific, which differ strikingly from those of its ancestral habitat, the Mediterranean Sea, may determine the success with which it is able to extend its range northward.

**Hypothesis**

My hypothesis is that the invasive species, while successful in the Mediterranean-like habitats of Southern California, will be limited in its further northern spread because it is less adapted to the highly variable salinities and low habitat temperatures of the northeastern Pacific.
**Genetics vs. Phenotypic Plasticity**

The goal of my studies of field populations and specimens subjected to acclimation (4 weeks) at different temperatures and salinities was to resolve ambiguities about the underlying basis—genetics or phenotypic plasticity (acclimatization ability)—of the observed differences in enzymatic activity between these species.

Since enzymatic activity appears to be a key indicator of survival, enzyme activity measurements made on field-collected and laboratory-acclimated specimens were a primary element in my studies. The metabolic capacities of organisms, as indexed by the levels of activity of ATP-generating enzymes, often provide important insights into an organism’s physiological status (Dahlhoff, 2004). Thus, the metabolic capacity of an organism is closely related to its ability to synthesize ATP: the cell’s ‘energy currency.’ Levels of ATP-generating enzymes in an organism’s tissues are determined by intrinsic genetic characteristics—the inherent catalytic efficiency of the enzyme plus the basal level of gene expression for the enzyme—and environmental factors like ambient temperature and salinity, which can alter gene expression and, thereby, enzyme levels. The ability to maintain optimal levels of enzymatic activity in the face of environmental change may determine whether a species can succeed in an altered environment.

My laboratory acclimation experiment was designed to determine whether the invasive species is capable of up-regulating enzymatic activities to offset the effects of reduced temperatures on metabolism. The acclimation experiments were also intended to reveal if adaptation to reduced salinity leads to alterations of metabolic capacity. The salinity of the Mediterranean Sea is slightly higher and much more stable than the salinity of near-shore waters of the Eastern Pacific. Thus, it is possible that the invasive species
is genetically adapted to a set of salinity conditions that differ significantly from those it
will face if it were to extend its range northward, where salinities are generally lower and
more variable. These studies of salinity acclimation may provide important predictive
insights into the further northward spread of *M. galloprovincialis*. The variable salinities
that characterize near-shore waters off Northern California, Oregon and Washington,
especially during winter run-off periods, may preclude a significant northward range
extension of the invasive species.

**Experimental Rationale**

The rationale for this study is foundationally based on an innovative series of
studies in the past several decades that have recognized the pertinence of using
physiological mechanisms to explain species range shifts in response to environmental
change. While many distributional shifts in marine species have been correlated with
changes in abiotic factors such as temperature, the physiological mechanisms behind
these changes have yet to be determined. At the same time, a holistic understanding of
these species shifts requires identification of the direct effects of abiotic factors on
cellular and organismal responses to external perturbations. Thus, physiological research
has the capacity to elucidate the processes underlying large-scale changes in ecological
systems. The blue mussel species complex provides a model system for comparative
studies of the physiologies of closely related native and invasive species and their hybrids
because the evolutionary relatedness of *Mytilus* species allows for adaptive physiological
differences to be discerned in the absence of confounding phylogenetic effects.

As succinctly outlined by Somero (2011), using multi-level physiological analysis
over multiple time frames is crucial to our understanding of global change-induced
biological shifts in a number of ways: First, distributional pattern shifts may be explained by the physiological limits of an organism. Knowing the lethal and sub-lethal abiotic stresses of a species may help predict the future shifts in biogeographic patterning in response to global change. Second, comparative physiological studies of different taxa can shed light on the vulnerabilities of species and ecosystems to global change. Characterizing the relative sensitivities of native and invasive congeneric species to environmental changes may help predict the success of the invasive species in a changing climate. Third, physiological studies help characterize acclimatization potential of organisms to environmental change. The potential for acclimatization based on adaptive phenotypic plasticity may be crucial in the short-term, before long-term genetically based adaptation can take place. Fourth, physiological studies of populations give insight into the fragility of these populations when exposed to changes in abiotic conditions. Fifth, physiological studies shed light on whether adaptive genetic change resulting in increased physiological fitness can keep pace with global change. The potential for acclimatization and evolutionary adaptation to environmental changes may ultimately determine whether a species survives or dies in its changing environment (Somero, 2011).

A meta-analysis incorporating physiological studies such as mine with broader ecological and oceanographic phenomena has the potential to enlighten marine scientists on the mechanisms underlying observed climate change-induced alterations in marine ecosystems and provide stronger predictive tools for the fate of these ecosystems in a changing ocean (Somero, 2011).
Chapter 2: Background

Evolutionary History of the Mytilus Species Complex

The *Mytilus* blue mussel complex consists of three species: *Mytilus galloprovincialis*, *M. trossulus*, and *M. edulis* (Linnaeus 1758) (Figure 2.1). *Mytilus trossulus*, a native of the North Pacific, is the ancestral species.

A trans-Arctic migration of the species occurred approximately 3.5 million years ago, leading to the establishment of populations in the North Atlantic (Vermeij, 1991). The advance of glaciers gave rise to *M. edulis*, via allopatric speciation. *M. edulis* subsequently spread throughout the North Atlantic and Mediterranean Sea. Mediterranean populations of *M. edulis* became geographically isolated approximately 2 million years ago (Seed, 1992) and gave rise to *M. galloprovincialis* (Barsotti and Meluzzi, 1968).

The North Pacific coast is characterized by cold water, low coastal salinity, large tidal range, and high seasonal variability (Mann and Hamilton, 1995), while the waters of
the Mediterranean are warmer, more saline, have a smaller tidal range, and lower seasonal variability (Thunell, 1979). Since *M. galloprovincialis* and *M. trossulus* have evolved in distinct abiotic environments, natural selection has differentially shaped the physiologies of these two species. Thus, the blue mussel species complex represents an excellent experimental system for discerning adaptive physiological differences between native and invasive species.

Because the two species are ‘cryptic,’ i.e., they cannot be distinguished on the basis of morphology, molecular forensic analysis was necessary in my studies of field populations to genotype all specimens (Braby and Somero, 2006a).

**Invasion of M. galloprovincialis: Biogeographic Patterning and Ecological Consequences**

Over the past century, *M. galloprovincialis* successfully established invasive populations in California (McDonald and Koehn, 1988), Japan, and South Africa (Daguin and Borsa, 1999). In each instance, it was transported from the Mediterranean in the ballast water of ships (Carlton and Geller, 1993). Because the invasion of *M. galloprovincialis* is globally widespread and has caused cumulative ecological damage—despite being less ‘visible’ because it replaces morphologically similar mussels—it has earned its dubious distinction as being named one of the "100 worst invasive species" in the world (Lowe et al., 2000).

*M. galloprovincialis* is a major space-occupying species and can drastically restructure marine communities, including causing the local extirpation of native species (Branch and Steffani, 2004; Robinson et al., 2005). However, the ecological damage caused by *M. galloprovincialis* has been less visible, destructive, and economically costly
than other invasive species. Thus, it is relevant and pertinent to ask how ‘bad’ of an invasive species *M. galloprovincialis* is before designing appropriate management policies.

After *M. galloprovincialis* was introduced to Southern California by the ballast water of ships (Geller et al., 1994) in the early twentieth century, it rapidly spread 1100 km northwards and became a biofouling species in many harbors. However, because *M. galloprovincialis* and *M. trossulus* are cryptic species, the invasion was not discovered until allozyme studies were performed on what was thought at the time to be different populations of *M. edulis* (McDonald and Koehn, 1988). The northward expansion of *M. galloprovincialis* has reflected changes in surface seawater temperature during the past several decades. The warming associated with the 1997-98 El Niño allowed *M. galloprovincialis* to expand north of Cape Mendocino (Rawson et al., 1999) and establish populations in Humboldt Bay (Hilbish, personal observation). More recently, the abundance of *M. galloprovincialis* has decreased over the northern extreme of its range. This decrease appears to be due to reductions in sea surface temperature caused by shifts in the Pacific Decadal Oscillation (Hilbish et al., 2010).

In addition to being ecologically invasive, *M. galloprovincialis* is also a source of ‘genetic pollution’ to the native mussel species where they co-occur (Hilbish et al., 2010). Hybridization and introgression can lead to homogenization or replacement of native genotypes, threatening the genetic integrity of *M. trossulus*. In contrast to the invasive success of the Mediterranean mussel, neither *M. trossulus* nor *M. edulis* has established invasive populations (Seed, 1992; Daguin and Borsa, 1999; Wonham, 2004).
In California, *M. galloprovincialis* has been documented as exhibiting competitive dominance over *M. trossulus* where they co-occur, as it is a superior space competitor and can reduce the growth and survival of the native species through indirect exploitation mechanisms (Shinen and Morgan, 2009). The invasive mussel’s competitive advantage is likely to be stronger at sites where abiotic conditions favor its growth and survival over that of the native (i.e., in warmer waters). A recent study by Braby and Somero (2006a) revealed that salinity might be just as important as temperature in influencing the relative abundances of the two species in the hybrid zone. In fact, the distribution patterns they found suggest that salinity may be more important than temperature as a survival indicator, since the abundance of *M. trossulus* correlated positively with low salinity in sites within the San Francisco Bay, CA, USA.

**Physiological Rates and Temperature Compensation**

Previous physiological studies have shown that *M. galloprovincialis* and *M. trossulus* have distinct thermal optima and thermal tolerances due to evolution under different abiotic conditions. All physiological studies of the two mussel congeners to date indicate that *M. galloprovincialis* is better adapted to warmer temperatures than *M. trossulus* (Lockwood and Somero, 2011). These findings confirm well-established conclusions that species adapted to different thermal environments possess metabolic characteristics that predispose them to function optimally within a particular thermal window that matches their native habitat (Hochachka and Somero, 2002; Pörtner, 2002). Since temperature controls the rates of metabolic reactions (Hochachka and Somero, 2002) and the two are positively correlated, cold-adapted species have been found to exhibit temperature compensation, whereby their metabolic rates are intrinsically higher.
than those of warm-adapted organisms when compared at common-garden (controlled) conditions. Temperature compensatory effects have been found in cardiac function and metabolic rates of *M. trossulus*. The resting heart rate of *M. trossulus* is 1.5-times faster than that of *M. galloprovincialis* at common-garden conditions (Braby and Somero, 2006b). Therefore, at low temperatures, the native cold-adapted species sustains significantly higher rates of cardiac function than the invasive warm-adapted species.

Temperature compensatory effects were also found in a study by Lockwood and Somero (2011) comparing enzyme activities in *M. galloprovincialis* and *M. trossulus* for CS and MDH in a common-garden laboratory acclimation. After acclimation, *M. trossulus* had significantly higher enzyme activities for both enzymes than *M. galloprovincialis* (1.7-fold higher MDH activity and 1.5-fold higher CS activity). They also found field-acclimatized native and invasive mussels from Bodega Bay, CA (in the hybrid zone) exhibited significant species-level differences in enzyme activities. Field-acclimatized hybrids had intermediate enzyme activities between the two species. Higher MDH and CS activities in *M. trossulus* suggest that it has an overall higher metabolic rate and therefore has greater maintenance energy costs than *M. galloprovincialis* (Lockwood and Somero, 2011).

The differences in physiological rates between the two species could be due to two mechanisms: either evolved or developmentally induced differences in gene expression that lead to differences in concentrations of metabolic enzymes, or differences in amino acid sequences of the orthologous enzymes that affect the kinetics of the enzyme-catalyzed reactions (Lockwood and Somero, 2011). The second mechanism is supported by studies of Fields et al. (2006), who found that the temperature compensation
in *M. trossulus* might be due entirely to intrinsic differences in catalytic efficiency ($k_{\text{cat}}$). A single amino acid difference between recombinant forms of cytosolic MDH (cMDH) in orthologous genes in *M. trossulus* and *M. galloprovincialis* is sufficient to cause significantly higher $k_{\text{cat}}$ in *M. trossulus* than its congener, a 2.2-fold difference. The discrepancy in ratios of the two species (*M. trossulus*/*M. galloprovincialis*) between MDH activities in cell tissues (1.4 for field-acclimatized mussels and 1.7 for laboratory-acclimated mussels) and the intrinsic rate of function per active site (2.2) suggests that *M. trossulus* may have lower concentrations of MDH in its tissues. Other biochemical and molecular differences related to thermal tolerance have also been observed in the two congeners. For example, the cMDH ortholog of *M. trossulus* has lower substrate binding ability at high temperatures than the cMDH of *M. galloprovincialis* (Fields et al., 2006). Thus, despite having a higher intrinsic catalytic activity, at temperatures in the upper environmental range of the species, the enzyme of *M. trossulus* may function poorly because of failure to bind its substrate effectively. The higher thermal tolerance of the invasive species may also be due in part to its enhanced ability to induce changes in the expression of particular genes and proteins in response to acute heat stress (Lockwood and Somero, 2011).

Taken together, these data predict that *M. galloprovincialis* will continue to be the dominant blue mussel species along the warmer range of the California coast. Because the northern biogeographic limit of *M. galloprovincialis* is not stable, but rather shifts to lower or higher latitudes in concert with oceanographic variability (Hilbish et al., 2010), it is important to further characterize the physiological constraints of *M.*
galloprovincialis, related to its sensitivity to cold temperatures and low salinities (Lockwood and Somero, 2011).
Chapter 3: Materials and Methods

To examine differences in the species’ responses to temperature and salinity in both field-acclimatized and laboratory-acclimated specimens, I exploited the tools of molecular population genetics to distinguish between species and the methods of enzyme biochemistry to characterize the metabolic capacities of the species and their hybrids. The specific methodologies are as follows:

Population Genetics Study

To assess the current biogeographical distribution of *Mytilus trossulus*, *Mytilus galloprovincialis*, and their hybrids, I sampled blue mussels from six sites within the hybrid zone in California, which lies between Monterey Bay and Cape Mendocino (36.6°N - 40.5°N) (Braby and Somero 2006a). These sites were selected to make data longitudinally comparable to Braby and Somero’s study (2006a). The Monterey Bay sample sites were Monterey Harbor (MM) and Moss Landing-South (MLS). The San Francisco Bay sites are Palo Alto, San Leandro, Lake Merritt, and San Rafael.

*Figure 3.1:* Map of 2008 sampling sites in the mosaic-hybrid zone. The Monterey Bay sites are Monterey and Moss Landing-South. The San Francisco Bay sites are Palo Alto, San Leandro, Lake Merritt, and San Rafael.
Francisco Bay sample sites were San Rafael (SR), Lake Merritt (LM), San Leandro (SL), and Palo Alto (PA) (Figure 3.1).

Approximately 40-50 mussels randomly sampled from each site were frozen immediately on dry ice to preserve their field-acclimatized enzyme levels and then stored in a cryogenic freezer (-80°C). Animals of similar size (3-5 cm) were collected to avoid potential artifacts from the influence of size on enzyme activity.

All specimens were genetically identified following protocols of Braby and Somero (2006a). DNA isolation from foot tissue used membrane spin columns (Macherey-Nagel, Nucleospin DNA extraction kit, Easton, PA, USA), and Proteinase K tissue digestion [10 mmol l\(^{-1}\) Tris-HCl pH 8, 1 mmol l\(^{-1}\) EDTA, 0.3% Tween, 0.3% nonylphenylpolyethyleneglycol (Sigma, IGEPAL CA-630), 0.03 U µl\(^{-1}\) Proteinase K; 55°C for 12 h, 98°C for 10 min]. Polymerase chain reaction (PCR) methods for distinguishing the two species involved two species-specific loci: the byssal thread protein (Glu-5') and the internal transcribed spacer region of ribosomal DNA (ITS) (Braby and Somero, 2006a). Species were distinguished based on characteristic base pairs using published primer sequences as described by Braby and Somero (2006a).

**Enzymatic Indictors of Metabolic Capacity**

To measure the metabolic enzyme activity of the two species and their hybrids as an indicator of their physiological condition, I measured activities in adductor muscle tissue of two enzymes that are quantitative indicators of activities of pathways of ATP generation, citrate synthase [CS; EC 4.1.3.7; citrate: oxaloacetate-lyase (CoA-acetylating)] and malate dehydrogenase (MDH; EC 1.1.1.37, L-malate: NAD+ oxidoreductase). CS is a tricarboxylic acid (TCA) cycle enzyme critical for aerobic
production of ATP. MDH is also involved in the TCA cycle and, in addition, is essential for anaerobic metabolism in bivalves when they experience routine environmental hypoxia. MDH exhibits adaptive variation related to temperature in most marine invertebrates (Dahlhoff and Somero, 1991; Dahlhoff and Somero, 1993; Fields et al., 2006). These two enzymes exist in all bivalves and are thus ideal for comparisons across species.

To prepare tissue homogenates for enzyme assays, approximately 0.5 g of adductor muscle tissue was dissected on ice from individual frozen mussels. Tissue was homogenized in ice-cold potassium phosphate buffer (50 mM, pH 6.8 at 4°C) as described by Fields et al. (2006). The homogenate was centrifuged at 20,000 g at 4°C for 5 minutes and the supernatant was removed and used to assay both MDH and CS.

Spectrophotometric (colorimetric) analysis using a temperature-controlled Shimadzu spectrophotometer was employed to measure rates of MDH and CS. The assay procedures followed those of Kawall et al. (2002).

**Laboratory Acclimation Study**

**Temperature.** I acclimated groups of 200 mussels collected from sites that are known to contain either *M. trossulus* (Boiler Bay, Oregon, 44.83°N, 124.06°W), *M. galloprovincialis* (Santa Barbara Harbor, California, 34.24°N, 119.41°W), or hybrids (San Rafael, California, 37.70°N, 122.19°W) to temperatures of 20°C and 10°C for a period of 4 weeks. I selected acclimation temperatures that are approximately 5°C above and below the hybrid zone averages in order to allow acclimation and avoid inducing acute stress responses. Four weeks is ample time for acclimatory effects to be completed in these
species (Braby and Somero, 2006b). Recirculating seawater aquaria whose temperatures were controlled by either a refrigeration system or heating element were used.

**Salinity.** Over the same 4 weeks, I acclimated approximately 200 specimens from the same collection sites used for the temperature acclimation experiments to two salinities, full-strength seawater (32 ppt) and brackish water (25 ppt). The 32 ppt tank was a flow-through tank with water incoming from Monterey Bay (ambient sea temperature: 14-16°C, ambient salinity: ~32 ppt). The 25 ppt acclimation was conducted in a recirculating temperature-controlled aquarium held at 15°C.

Specimens of similar size (3-5 cm) were used in order to reduce potential influences of animal size on metabolic capacity. Genotyping of the specimens was done using the same methods for the field-acclimatized mussels to ascertain if each individual was a native, invasive, or hybrid. The metabolic activity of MDH was measured using the same spectrophotometric methods as above to see if the species differed in their responses to changes in ambient temperature and salinity. CS activity was not measured for the acclimation study because my studies of field-acclimatized mussels showed CS and MDH to exhibit similar patterns related to species-specific differences.

During acclimation, records of mortality in the tanks were kept to determine if the species differed in tolerance of temperature or salinity. These data are reported as survivability (% surviving). Mussels were fed Shellfish Diet 1800® 3 days a week and checked for mortality on the same days. Tanks were cleaned once a week to hinder disease-related mortality.

*Statistical Analysis*
For the field study, I compared MDH and CS activities between *M. galloprovincialis*, *M. trossulus*, and the hybrids (n = ~40-50 for each species) using a one-way analysis of variance (ANOVA) and Tukey’s post-hoc test for group comparisons.

I performed a power analysis in designing the acclimation experiment to determine the appropriate sample sizes. Following the acclimation, changes in MDH activities over time were tested for significance at the 5% level using one-way analysis of variance (ANOVA) and Tukey’s post-hoc tests for group comparisons. Mean enzymatic activities were calculated for each species and are represented ± standard error (± SE). Pertinent Tukey’s post-hoc test results are presented in Appendix 2.
Chapter 4: Results

Field Study of Biogeography

The current biogeographic distributions of the two West Coast blue mussels do not follow a uniform gradient with latitude (Figure 4.1). This finding is consistent with the mosaic-hybrid zone model, which posits that local variations in temperature and salinity conditions determine species distributions. Hybrids were not present at all sites in the mosaic-hybrid zone and were less abundant than the frequencies recorded by Braby and Somero (2006a) shown in Figure 4.2. Data was not available for two sites (LM and MLS) in the 1990s.

Figure 4.1: Blue mussel species distribution at the six 2008 sampling sites. From North to South, they are San Rafael (‘SR’), Lake Merritt (‘LM’), San Leandro (‘SL’), Palo Alto (‘PA’), Moss Landing South (‘MLS’), and Monterey (‘MM’). Percentage of each species at each site is represented by *M. trossulus* in white, hybrids in gray, and *M. galloprovincialis* is in black.

<table>
<thead>
<tr>
<th>Site</th>
<th>SR</th>
<th>LM</th>
<th>SL</th>
<th>PA</th>
<th>MLS</th>
<th>MM</th>
</tr>
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<tbody>
<tr>
<td><strong>SR</strong></td>
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<td><strong>LM</strong></td>
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<tr>
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<tr>
<td><strong>MLS</strong></td>
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<tr>
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<td></td>
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</tr>
</tbody>
</table>

SR = San Rafael
LM = Lake Merritt
SL = San Leandro
PA = Palo Alto
MLS = Moss Landing-South
MM = Monterey

Summer 2008
**Figure 4.2a:** Blue mussel species distribution in the 1990s (Braby and Somero, 2006a).

**Figure 4.2b:** Blue mussel species distribution in the early 2000s (Braby and Somero, 2006a).
In the early 2000s, *M. trossulus* was found to comprise a greater percentage of the population at Monterey Bay and San Francisco Bay sites than it did in the 1990s. This was an unexpected result at the time because *M. galloprovincialis* had consistently spread northwards since its arrival in California (Braby and Somero, 2006a). The data from the present study show that in 2008, species proportions in Monterey Bay and San Francisco Bay were more consistent with data from the 1990s than with data from the early 2000s. The 2008 field collection data indicate that for five of the six sampling sites in Monterey Bay and San Francisco Bay, *M. galloprovincialis* increased in relative abundance (Moss Landing-South being the exception, where *M. trossulus* became relatively more abundant than *M. galloprovincialis*) compared to the early 2000s data. Whereas hybrids were observed at all six sites in the hybrid zone in the early 2000s, in 2008, they were not found in either Moss Landing-South (Monterey Bay) or at Lake Merritt (San Francisco Bay).

Interestingly, the native species was not found at San Rafael in 2008 despite having been present at that site since sampling began in the 1990s. Instead, their population percentage was 'replaced' by hybrids. The native mussels were much less abundant in the sampling sites in San Francisco Bay (SR, LM, SL, PA) in 2008, and entirely absent from the northernmost San Francisco Bay sites (SR, LM).

Overall, *M. galloprovincialis* increased in percentage of total population across all six sites from the early 2000s to 2008.

**Correlating Species Abundances with Salinity and Temperature**

At the five sample sites where temperature data were available (Table 4.1a), *M. galloprovincialis* abundance was positively correlated with salinity but the only
significant correlation was with minimum site salinity \((R^2 = 0.88, p<0.05)\) (Table 4.1b).

<table>
<thead>
<tr>
<th>Habitat category</th>
<th>SR</th>
<th>SL</th>
<th>PA</th>
<th>MLS</th>
<th>MM</th>
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<tbody>
<tr>
<td>Temperature (average)</td>
<td>E</td>
<td>I</td>
<td>E</td>
<td>I</td>
<td>O</td>
</tr>
<tr>
<td>16.5</td>
<td>16.7</td>
<td>17</td>
<td>14.6</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>Salinity (average)</td>
<td>22.7</td>
<td>26</td>
<td>20.1</td>
<td>22.9</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 4.1a: 2001-2003 temperature (ºC) and salinity (ppt) data by site. Habitat category distinguishes estuarine (E), oceanic (O), and intermediate (I) sites.

<table>
<thead>
<tr>
<th></th>
<th>M. trossulus</th>
<th>Hybrid</th>
<th>M. galloprovincialis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(R^2) Slope P</td>
<td>(R^2) Slope P</td>
<td>(R^2) Slope P</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>0.15</td>
<td>-8.20</td>
<td>0.52</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.03</td>
<td>-1.02</td>
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</tr>
<tr>
<td>Minimum</td>
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<td>1.43</td>
<td>0.90</td>
</tr>
<tr>
<td>Salinity</td>
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<td></td>
</tr>
<tr>
<td>Average</td>
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<td>-1.92</td>
<td>0.59</td>
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<tr>
<td>Maximum</td>
<td>0.00</td>
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<td>0.93</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.38</td>
<td>-2.84</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 4.1b: Blue mussel species correlation of genotype distribution with site characteristics. Correlations with \(R^2\) of 0.5 or more and significant \(P\) values (<0.05) are in bold type.

No significant correlation between the percentage of the \(M.\) galloprovincialis genotype and temperature (as an average, maximum, or minimum) was found. Neither did \(M.\) trossulus abundance correlate with temperature. \(M.\) trossulus and hybrid frequencies negatively correlated with measurements of salinity, but the trends were not statistically significant.

Field Study of Enzyme Activity
In my preliminary studies in the summer of 2008 in which I used field-acclimatized specimens, I observed significantly higher levels of CS and MDH activity in tissues of the native species, *M. trossulus*, than in the invasive, *M. galloprovincialis* (P<0.05) (Figures 4.3 and 4.4). The hybrids had the same levels of CS activity as *M. galloprovincialis* (Figure 4.3) and intermediate levels of MDH activity between the parental species (Figure 4.4).

**Figure 4.3:** Pooled average rates of CS activity in field acclimatized mussels: M. galloprovincialis (Gallo), hybrids, and M. trossulus (Tross) in International Units per gram fresh weight (I.U./gfw). There are significant differences between Gallo and Tross, and Hybrid and Tross (p < 0.05).
**Laboratory Acclimation Study**

It is unknown why after three weeks of temperature and salinity acclimation (Time Point 3) there was an anomalous peak in enzyme activity for all species and tank treatments. While it could be a timed up-regulation in enzymatic activity to compensate for new stressful abiotic conditions, because the control tank also exhibited the same significant peak, it was likely an experimental error, possibly from using the wrong dilution factor for the homogenization buffer before assays. Therefore, the Time Point 3 data were excluded from statistical analysis. After excluding Time Point 3 from statistical analysis of the acclimation results, *M. trossulus* showed no significant alterations in enzyme activity over the course of acclimation pooled across all tank treatments (Figure 4.5a).

**Figure 4.4:** Pooled average rates of MDH activity in field-acclimatized mussels: *M. galloprovincialis* (Gallo), hybrids, and *M. trossulus* (Tross). There are significant differences between both species and the hybrids (p < 0.05).
*M. galloprovincialis* similarly had but a minimal response to acclimation to the imposed temperature and salinity conditions. With the exception of Time Point 2, at which MDH activities were higher than at other acclimation times, there were no significant differences in MDH activity (Figure 4.5b).

In fully acclimated mussels (Time Point 4), species-level enzyme activity differences remained significant with *M. trossulus* maintaining significantly higher MDH activity than *M. galloprovincialis* (Figure 4.6). Hybrids had significantly higher MDH activity than either parental species after acclimation (Figure 4.6).

**Figure 4.5a,b:** Average MDH activity in acclimated blue mussels excluding Time Point 3 (TP3) because of anomalous results. a) For *M. trossulus* (Tross), there are no significant differences between the time points. b) *M. galloprovincialis* (Gallo) had significantly higher MDH activity at TP2 (after 2 weeks of acclimation).
Relative to enzyme activity values measured in field-acclimatized specimens, *M. galloprovincialis* and *M. trossulus* down-regulated enzyme activity during acclimation (Figure 4.7). In contrast, in the pooled data, hybrids had significantly higher MDH activity after acclimation compared to field acclimatized hybrids (Figure 4.7).

**Figure 4.6:** Average rates of MDH activity in fully acclimated (Time Point 4) mussels: *M. galloprovincialis* (*Gallo*), hybrids, and *M. trossulus* (*Tross*) pooled across the 4 treatment tanks. There are significant differences between both species and the hybrids (*p* < 0.05).
Mussel Mortality

*M. trossulus* had increasing mortality rates in the ‘warm’ (20°C) tank throughout acclimation, peaking after two weeks (Time Point 2) with mortality greater than 40% (Figure 4.8a). *M. trossulus* exhibited increasing mortality rates in the ‘dilute’ (25 ppt) tank as well, peaking at around 10% mortality at week 3. It had no mortalities in the ‘cold’ (10°C) tank and insignificant total mortality in the control (32 ppt) tank.
Figure 4.8a,b,c: Survivorship (in percent) of mussels during 4-week acclimation. Mortality was measured every other day except weekends during acclimation. Blue line is 10°C tank (#1), red is 20°C tank (#2), yellow is 25ppt tank (#3), and green is control tank (#4). a) *M. trossulus* from Oregon (>90% *M. trossulus*), b) an unknown mixture of *M. galloprovincialis* and hybrids from San Rafael (46% hybrids and 54% *M. galloprovincialis* based on 2008 field study), and c) *M. galloprovincialis* from Santa Barbara (>90% *M. galloprovincialis*).
M. galloprovincialis showed both ‘cold’ and ‘warm’ water tolerance, suffering less than 10% mortality in each tank (Figure 4.8c). It had no mortalities in the ‘dilute’ tank, and less than 10% total mortalities in the control tank.

Mortality of hybrids that likely were present in the San Rafael tank (Figure 4.8b) could not be determined because post-mortem analysis was not feasible due to how rapidly tissues decayed in the dead mussels. Genetic analysis cannot be performed on decomposing tissues.
Chapter 5: Discussion

My hypothesis—that *M. galloprovincialis* will be limited in its further northern range expansion because it is less adapted to the highly variable salinities and low habitat temperatures of the northeastern Pacific—is still valid based on the experimental results of this study. However, my initial expectations were sometimes stretched by the actual data, causing me to realize that trying to isolate two key, determinant variables out of a complex array of interacting factors is perhaps overly simplistic.

My studies of field-acclimatized populations within the hybrid zone concurred with established findings that *M. trossulus* exhibits temperature compensation effects, indicating it is better suited to cold environments than the invasive species and may, thus, win out in northern latitudes. However, my biogeographical data showing an increase in *M. galloprovincialis* at the northernmost field collection sites since the early 2000s was an unanticipated finding based on this previous result.

My laboratory acclimation experiment revealed that genetic adaptation to the temperature and salinity conditions of their ancestral habitats is likely the basis for the observed significant differences between the metabolic activities of *M. galloprovincialis* and *M. trossulus*, since those significant differences were maintained even after temperature and salinity acclimation.

These findings are discussed in more detail below:

**Field Study of Biogeography**

Comparing the blue mussel genotype distributions from the 1990s, early 2000s, and 2008, there are no obvious trends in distribution changes through time. It is clear, however, that local populations change, sometimes drastically, over time scales of
decades or years. Overall, in 2008 the abundance of *M. galloprovincialis* increased at most sites relative to the early 2000s. The single exception is a site near the southern edge of the hybrid zone (Moss Landing-South). The overall increased abundance of the invasive did not reflect a strict latitudinal relationship, but rather variation that may reflect local abiotic conditions, as the mosaic-hybrid zone predicts. Based on this evidence of an overall increase in *M. galloprovincialis* abundance in the hybrid zone, the invasive may be moving northward and the hybrid zone concomitantly expanding to the north as well. In addition, *M. trossulus* was much less abundant at the northernmost field collection sites in 2008 compared to the early 2000s. To verify that this observed variation is valid versus an anomaly caused by sampling effect, repeatability of the data is recommended. However, if these changes are not the result of confounding factors, this finding could suggest there is gradual competitive success of *M. galloprovincialis* at northern sites. This may have resulted from adaptation or acclimation to abiotic conditions at these sites or changes in local abiotic conditions that are favoring *M. galloprovincialis*. As expected on the basis of the mosaic-hybrid zone model, the genotype composition at a given location is unpredictable based on latitude alone.

**Correlating Species Abundances with Salinity and Temperature**

It is not clear from the results correlating *Mytilus* species distribution with temperature and salinity which abiotic factor is of greater importance in governing the distribution patterns of the genotypes. However, the significant correlation between *M. galloprovincialis* abundance and salinity (as a minimum) was also found by Braby and Somero’s (2006a) field study. Their three years of sampling show that frequency of *M. trossulus* was negatively correlated ($R^2 = 0.60$) with salinity, whereas frequency of *M.
*M. galloprovincialis* \( (R^2 = 0.76) \) was positively correlated with salinity. Thus, the salinity-correlated distribution patterns indicated that *M. galloprovincialis* was found at more saline sites and that the native congener is more abundant at sites with low salinity.

For temperature, Braby and Somero (2006a) found that the percentage of *M. trossulus* found across all sites was positively correlated with temperature \( (R^2 = 0.60) \), whereas the frequency of *M. galloprovincialis* was negatively correlated with temperature \( (R^2 = 0.50) \). These data suggest that, whereas *M. galloprovincialis* is physiologically adapted to higher temperatures than *M. trossulus*, differences in ambient salinity may play a more important role in establishing biogeographic patterning than variations in temperature.

In my studies, despite an absence of clear trends in temperature and salinity effects on distributions across all six sites, there were patterns within the two bay systems. All San Francisco Bay sites (SR, SL, and PA) had significant *M. trossulus* populations in the early 2000s, where conditions have been more estuarine. In Monterey Bay, there was a similar pattern with more *M. trossulus* at MLS compared to MM. The large spatial and temporal variation found in the frequencies of the three genotypes (two parental species plus hybrids) illustrates the dynamic nature of this species invasion and suggests that future shifts in the biogeography of the invasive species will be governed by complex, interacting influences of both temperature and salinity.

**Field Study of Enzyme Activity**

The significantly higher MDH and CS activity in *M. trossulus* compared to *M. galloprovincialis* at all sites where they co-occur suggests that the native species can sustain a higher rate of ATP production than the invasive, under common temperature
exposures. The higher levels of enzymatic activity in the native species are consistent with it being more cold-adapted. Thus, the higher intrinsic levels of ATP-generating enzymes in the native species would enable it to sustain its metabolic rate at low temperatures more successfully than the Mediterranean congener, therefore making it better adapted to colder climates. However, data from field-acclimatized specimens do not permit a distinction to be drawn between inherent species-specific differences in gene expression and differences due to acclimatization history in the field.

My data suggest that *M. trossulus* is the more cold-adapted species, a finding that is consistent with earlier studies of the two congeners. In 1996, Hofmann and Somero found evidence that *M. trossulus* was more cold-adapted than *M. galloprovincialis* by studying the induction temperatures of heat-shock protein synthesis. Braby and Somero (2006b) showed that heart failure occurred at a higher temperature in *M. galloprovincialis* than in *M. trossulus*. They also found that the heart rate of *M. trossulus* was significantly higher than that of *M. galloprovincialis*, since it is adapted to colder temperatures, and the heart rates of the hybrids were intermediate between those of the parental species (Braby and Somero, 2006b). Fields et al. (2006) showed that the functional properties and heat stabilities of cytosolic malate dehydrogenases (cMDH) of the two species differed as expected for cold- and warm-adapted species. The higher substrate turnover number (catalytic rate constant ($k_{cat}$)) observed by Fields et al. (2006) for the cMDH of *M. trossulus* may explain the higher tissue levels of MDH activity I observed in my studies. Thus, both the available data and my study argue for intrinsic, genetically based differences between the two species in thermal optima and thermal tolerance limits.
**Laboratory Acclimation Study**

After excluding the anomalous Time Point 3 data, the general lack of statistically significant differences in enzymatic activity during the four-week acclimation period suggests that the underlying basis for the observed differences in enzyme activity between the two species is genetic and not subject to effects from temperature- and salinity-acclimation. It is also possible that the acclimation period may not have been long enough to see major up- or down-regulation of enzyme rates, albeit the study of Braby and Somero (2006b) showed that this was a sufficient period to allow full acclimation of cardiac activity to occur. Acclimation of levels of enzymes would be expected to occur even more rapidly than such organ-level responses (Hochachka and Somero, 2002). Therefore, I conjecture that the lack of acclimation effects on enzymatic activity can be taken as an indication that fixed, genetically based differences between the two species account for the observed differences in levels of enzymatic activity in field populations.

**Mussel Mortality**

The survivability results from the acclimation experiment suggest that *M. trossulus* has a lower overall tolerance to laboratory holding than its congener, *M. galloprovincialis*. This result is in agreement with the findings of Braby and Somero (2006b), who noted lower survivorship in the native species relative to the invasive during acclimation to different temperatures and salinities. The results of Shinen and Morgan (2009) also show the relative robustness of *M. galloprovincialis* compared to *M. trossulus*. Since these previous studies suggest that *M. trossulus* has a lower survivability in laboratory settings than *M. galloprovincialis*, possibly due to susceptibility to disease,
this hinders my ability to use survivability data to help explain acclimation abilities of the
two species.

**Oceanographic Factors Influencing Species Ranges**

In a 2005-08 sampling period, Hilbish et al. (2010) found that the distribution
range of *M. galloprovincialis* and the hybrid zone rapidly shifted in the opposite direction
as expected in response to global warming. They sampled three of the same sites—San
Rafael, San Leandro, and Monterey—from 2005-2007, just 1-3 years before the 2008
sampling period for this study. A southward shift in *M. galloprovincialis* was found,
whereas the distribution of *M. trossulus* remained unchanged over same time period.
They found that the shift corresponded to decadal climate variation associated with the El
Niño/Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO). Since the
biogeography of the mussels was characterized in 1999, the PDO has gone from a warm
phase (dominated by frequent and large El Niño events), to a cold phase, with minimal
ENSO activity. The difference in SST between a warm and cold phase of the PDO is only
approximately 1°C. The warm phase of the PDO and ENSO may lead to warmer near-
shore sea surface temperatures (SSTs) from depressed upwelling. While global warming
predicts the poleward range expansion of the warm-adapted invasive species and
concurrent northward movement of the hybrid zone, decade-scale changes in SSTs
appear to play a large role in governing mussel distributions. In addition, local variations
in temperature and salinity (governed by precipitation patterns, coastal currents,
freshwater inputs from land, etc.) add further complexity to the biogeographic patterning
of the mussels. Predicting the further northern spread of the invasive blue mussel will
prove difficult in light of the remaining uncertainties regarding their ability to adapt to
lower and more variable salinity.

Analyses of these cause-effect linkages between effects of abiotic factors on physiology and biogeographic shifts demand a holistic, meta-approach that considers multiple, interacting physiological systems and abiotic variables.
Chapter 6: Conclusions and Policy Implications

Past research has elucidated the mechanisms behind thermal stress responses in marine organisms; however, less is known about these organisms’ long-term response to warming (Anestis et al., 2007). By studying the past and current distributional patterning of *Mytilus* species, the intention is to build a predictive model for how the hybrid zone will evolve over time in response to climate change. Climate change will likely alter the distributions of these two mussel species as well as their likelihood to hybridize in the future. Warming sea temperatures in northern latitudes appear to be making it easier for *M. galloprovincialis* to invade. Also, as warming temperatures threaten *M. trossulus*, it is easier for *M. galloprovincialis* to compete. However, the higher levels of rainfall to the north of *M. galloprovincialis*’ current biogeographic range could be an important factor in inhibiting its northward invasion. Even though lower salinities may not be immediately lethal to the invasive species, as my 4-week acclimation experiment revealed, the positive correlation between frequency of *M. galloprovincialis* across sites and ambient salinity found by Braby and Somero (2006a) is an indication that reduced salinity may provide the native species with a competitive advantage under these circumstances. Further studies of these species may shed more definitive light on the relative importance of these two major environmental factors, temperature and salinity, in governing the spread of the invasive species and thus allow a predictive mechanistic model to be developed. Having such a model would allow us to foresee how climate change is apt to differentially affect the abilities of the native and invasive mussel to compete in Eastern Pacific coastal ecosystems.
Although it might seem that these two closely related cryptic species could replace each other without overall effects occurring on the ecosystem, this is not the case. *M. galloprovincialis* grows faster than its congener and reaches significantly larger size at maturity (Braby and Somero, 2006a), hence the replacement of *M. trossulus* by *M. galloprovincialis* could lead to a more strongly mussel-dominated ecosystem. For this reason, being able to predict the further invasive success of *M. galloprovincialis* would also allow predictions of broader ecological changes as a result of climate change.

Having a bigger and faster growing mussel species take over could possibly alter the balance of other species within that marine environment. For instance, the invasive species has the potential to increase the food sources for consumers of marine mussels, such as sea otters. While this initial consequence may appear to be favorable, this boost in food sources may increase predator populations and could eventually lead to trophic cascades whereby lower levels of the food chain are affected.

While the ecological consequences of continued *M. galloprovincialis* invasion appear dismal for native species, potential economic benefits to humans may outweigh some of the costs of the invasion to society. The same physiological factors that make *M. galloprovincialis* superior over native species—robust growth and larger body size—make it attractive for mussel farming. In response to an increasing long-term trend in US per capita consumption of seafood, mariculture and aquaculture industries have spawned to support the growing demand for fresh mollusks. In fact, aquaculture is among the fastest-growing segments of the world food economy (Naylor et al., 2001), with mollusk farming alone generating over $100 million annually. The future economic outlook for commercialized mussel ocean mariculture is both strong and relatively secure because the
current supply side of fresh mollusks is fixed due to limits on wild harvest. Currently, most US mussel farms are located in Maine, where *M. edulis* is harvested. However, with increasing seafood demand and the robustness of *M. galloprovincialis*, it seems likely that the Mediterranean mussel is well suited to become the next farmed favorite. Logically, the commercial value and profitability potential of farmed *M. galloprovincialis* is greater than that of *M. trossulus* due to its faster growth rate and larger body mass. Thus, the replacement of *M. trossulus* with *M. galloprovincialis* represents a potentially economically viable and profitable shift. However, wherever *M. galloprovincialis* is grown in aquaculture, it poses clear potential threats of invading surrounding areas. In addition, the potential further northern spread of *M. galloprovincialis* in California in response to global warming as well as accidental introduction in coastal areas create an urgent need in the marine science community to continue to study the ecosystem-level effects of the invasion of *M. galloprovincialis* on the California coastline. Before commercial farming practices are put in place, a cost-benefit analysis should be conducted to quantify the positive and negative impacts this species will have on the ecosystem and to human activity and industry.

By mapping out the abiotic conditions that support invasion by *M. galloprovincialis* along the western US seacoast, such as high salinity and high temperatures, we may build predictive models for areas vulnerable to invasion by *M. galloprovincialis*. However, this study and others before it illustrate the complexity of studying physiological parameters as determinants of the future success of the invasive over the native.
Because of their accessibility and abundance, blue mussels are ideal organisms for studying invasions. By tracking the interactions between invasive and native species and understanding the physiological determinants of invasive success, we are advancing our scientific ability to construct models that may predict the further spread of invasive species. Being able to predict the ecological and financial implications of an invasion in a more reliable and timely manner would open up an array of species management options that might otherwise not be practical or plausible to implement if the invasion had reached a later stage with greater penetration. In the event of a severely crippling and costly invasion, such as the zebra mussel invasion, definitive, evasive action would have resulted in tremendous economic savings. Mechanistic predictive models have the inherent benefit of providing reliable data before invasions reach critical mass, thus enabling decision makers to make swift, sound trade-offs that can reduce ecosystem and economic damage. If invasive species were ranked on a scale from the extremely destructive, such as the zebra mussel, to the more benign, to the beneficial, *M. galloprovincialis* would most likely fall somewhere in the middle since its ecological impacts have been less visible despite its invasion being geographically widespread. One such beneficial invader is the Asian oyster, which positively impacts the environment by filtering out water pollutants more effectively than native oysters. In this case, since Asian oysters also grow faster and have stronger immunity to diseases than the native species, biologists are considering introducing these potentially advantageous invaders to Chesapeake Bay with the hope of significantly improving water quality (Ruesink et al., 2005). In contrast, the zebra mussel (*Dreissena polymorpha*) represents one of the most harmful and expensive invasive species in the world. Since invading US Great Lakes as
planktonic larvae, likely transported in the ballast water of trade ships (Holeck et al., 2004), it has overwhelmed pilings, docks, and underwater pipes, displacing many native species and costing millions of dollars. While thus far *M. galloprovincialis* has appeared to be a rather obsequious visitor—unlike the zebra mussel, which has created a wake of economic and ecosystem devastation that is likely to ripple on—there is still a clear benefit to monitoring the implications its arrival and possible replacement of *M. trossulus* might have. Thus, in summary, having a mechanistic predictive model in place will provide us with a broader range of more efficient possible responses to address the future effects of invasions by *M. galloprovincialis* as global warming progresses.
APPENDICES
APPENDIX 1

COORDINATES OF 2008 FIELD COLLECTION SITES

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<thead>
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<th>Site</th>
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<th>Longitude</th>
</tr>
</thead>
<tbody>
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</tr>
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<td>122.1041 W</td>
</tr>
<tr>
<td>Moss Landing</td>
<td>36.803656 N</td>
<td>121.793622 W</td>
</tr>
<tr>
<td>Monterey</td>
<td>36.609386 N</td>
<td>121.877517 W</td>
</tr>
</tbody>
</table>
**APPENDIX 2**

**STATISTICAL TABLES**

Pooled field-acclimatized mussels: MDH activity

<table>
<thead>
<tr>
<th>Tukey’s Test</th>
<th>hybrid vs tross</th>
<th>hybrid vs gallo</th>
<th>tross vs gallo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (P&lt;0.05)?</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Pooled field-acclimatized mussels: CS activity

<table>
<thead>
<tr>
<th>Tukey’s Test</th>
<th>hybrid vs tross</th>
<th>hybrid vs gallo</th>
<th>tross vs gallo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (P&lt;0.05)?</td>
<td>***</td>
<td>ns</td>
<td>***</td>
</tr>
</tbody>
</table>

Pooled laboratory-acclimated mussels: MDH activity

<table>
<thead>
<tr>
<th>Tukey’s Test</th>
<th>hybrid vs tross</th>
<th>hybrid vs gallo</th>
<th>tross vs gallo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (P&lt;0.05)?</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Field-acclimatized vs. laboratory-acclimated mussels: MDH activity

<table>
<thead>
<tr>
<th>Tukey’s Test</th>
<th>Field hybrid vs lab hybrid</th>
<th>Field tross vs lab tross</th>
<th>Field gallo vs lab gallo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sig. (P&lt;0.05)?</td>
<td>***</td>
<td>ns</td>
<td>*</td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY


